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RESEARCH NOTE / NOTA CIENTÍFICA

CANINE MULTICENTRIC LYMPHOMA-DERIVED *TRICHURIS VULPIS* HYPERINFECTION

HIPERINFECCIÓN POR *TRICHURIS VULPIS* DERIVADA DEL LINFOMA MULTICÉNTRICO CANINO

HIPERINFECÇÃO POR *TRICHURIS VULPIS* DERIVADA DE LINFOMA MULTICÊNTRICO CANINO

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Running Head: Canine lymphoma-derived *Trichuris vulpis* hyperinfection

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ABSTRACT

Trichuris vulpis (Roederer, 1761) is a geohelminth in the large intestine of wild carnivores, including domestic cats and occasionally humans. However, canine lymphoma, one of the most aggressive neoplasms in the small animal clinic, tends as a direct consequence of the loss of immunological response. Moreover, such parasite-related infections could develop into more serious conditions. In this study, we report a *T. vulpis* hyperinfection case in a canine affected by multicentric lymphoma. Physical examinations revealed lymphadenomegaly, haematochezia, and hind limb oedema. Despite the obtained treatment, the patient died. Macroscopic and microscopic examinations revealed congested lungs and legs, splenomegaly, lymphadenomegaly with leukocyte infiltration, and bloody large intestine mucosa with 528 adult *T. vulpis* specimens. Periodic coproparasitological examinations and specific antiparasitic drug applications are considered important, beyond preventive examinations for neoplasias, mainly in vulnerable patients such as older dogs.

Keywords: Canine lymphoma – hyper-infection – Trichocephalus – Trichuriasis

RESUMEN

Trichuris vulpis (Roederer, 1761) es un geohelminto del intestino grueso de los carnívoros silvestres, incluidos los gatos domésticos y ocasionalmente los humanos. Sin embargo, el linfoma canino, una de las neoplasias más agresivas en la clínica de pequeños animales, tiende a ser una consecuencia directa de la pérdida de la respuesta inmunológica. Además, estas infecciones parasitarias podrían derivar en afecciones más graves. En este estudio, informamos de un caso de hiperinfección por *T. vulpis* en un canino afectado por linfoma multicéntrico. Los exámenes físicos revelaron linfadenomegalia, hematoquecia y edema de las extremidades traseras. A pesar del tratamiento recibido el paciente falleció. Los exámenes macroscópicos y microscópicos revelaron pulmones y piernas congestionados, esplenomegalia, linfadenomegalia con infiltración de leucocitos y mucosa del intestino grueso sanguinolenta con 528 especímenes de *T. vulpis* adultos. Se consideran importantes los exámenes coproparasitológicos periódicos y la aplicación de fármacos antiparasitarios específicos, más allá de los exámenes preventivos de neoplasias, principalmente en pacientes vulnerables como los perros mayores.

Palabras clave: hiperinfección – Linfoma canino – Tricuriasis – Tricocefalia

RESUMO

Trichuris vulpis (Roederer, 1761) é um geohelminto do intestino grosso de carnívoros selvagens, incluindo gatos domésticos e ocasionalmente humanos. De outro lado, o linfoma canino, uma das neoplasias mais agressivas na clínica de pequenos animais, com uma consequência direta na perda da resposta imunológica. Esta condição tumoral relacionada ao parasitismo pode evoluir para condições mais graves. Neste estudo, relatamos um caso de hiperinfecção por *T. vulpis* em um canino afetado por linfoma multicêntrico. Os exames físicos revelaram linfadenomegalia, hematoquezia e edema de membros posteriores. Apesar do tratamento aplicado, o paciente morreu. Os exames

macroscópicos e microscópicos revelaram pulmões e pernas congestionados, esplenomegalia, linfadenomegalia com infiltração de leucócitos e mucosa do intestino grosso sanguinolenta com a observação de 528 espécimes adultos de *T. vulpis*. Exames coproparasitológicos periódicos e aplicações de medicamentos antiparasitários específicos são considerados importantes, além de exames preventivos para neoplasias, principalmente em pacientes vulneráveis, como cães idosos.

Palavras-chave: hiperinfecção – Linfoma canino – Trichocephalus –Trichuriasis

INTRODUCTION

Canine lymphoma is among the most aggressive neoplasms in the clinic of small animals with a direct consequence of losing the immunological response for affecting the cells of the lymphatic system (Bally *et al*., 2018) being responsible for immunity and defence. The pathological alterations of this neoplasm could be divided into multicentric, gastrointestinal, and mediastinal lymphoma, causing lymphadenomegaly, weight loss, lethargy, vomiting, diarrhoea, respiratory difficulty, and limb oedema (Fighera *et al*., 2020; Beraldo *et al*., 2020). Helminthic infection cases or patients with lymphoma are susceptible to an accelerated parasite load increase, allowing for the proliferation of infective adult forms as observed in the case of feline infections by *Aelurostrongylus abstrusus* (Railliet, 1898) and *Platynosomum illiciens* (Braun, 1901) associated with lymphoma (Riveros-Pinilla *et al.,* 2011: Terra *et al.,* 2015). *Trichuris vulpis* Roederer, 1761 is a geohelminth with macroscopically different morphological characteristics compared to other helminths (Ferraz *et al*., 2020). The anterior end of the animal is long and thin while the posterior part is wider and more robust. It lays mucilaginous bitamponate eggs, easily recognizable in laboratory diagnosis. This nematode infecting the large intestine of wild carnivores including domestic dogs and cats, opportunely affecting humans (Dunn *et al*., 2002: Areekul *et al*., 2010; Yevstafieva *et al.,* 2019). In

most cases, the infection is mild with no considerable clinical signs. However, in the case of intense infections, it could cause abdominal pain, rectal prolapse, bloody diarrhoea, dehydration, anaemia, and progressive weight loss (Acha & Szyfres, 2005). In this study, we report a *T. vulpis* hyperinfection case in a canine patient diagnosed with multicentric lymphoma.

CASE PRESENTATION

In March 2023, a 13-year-old female canine of an undefined breed was treated at the Veterinary Hospital of the *Universidade Federal Rural da Amazônia* (1° 27' 31" S 48° 26' 04.5" O) in the city of Belém-Pará, Brazil. The guardian provided the information that the patient has had progressive weight loss for four months coupled with a loss of appetite, and that he has previously taken antiparasitic medication without informing any name. The physical examination revealed lymphadenomegaly in the submandibular, popliteal, inguinal, and mammary lymph nodes as well as alopecia in the abdomen, in addition to a slight degree of dehydration, haematochezia, and oedema in the posterior limbs with difficulties in walking and alterations in cardiac auscultation. Therefore, we applied furosemide- and prednisolone-based treatment for oedema and requested hemocytometry, biochemistry, and oncological cytology of the lymph nodes.

In the blood count, we observed macrocytic anaemia with mild hypochromia and thrombocytopenia (160,000 mil/mm^3). The biochemical examination revealed an increased alkaline phosphatase level of 386.6 U/L (ref: 20–150 U/L). Despite the palliative treatment, the patient died 48 hours after the consultation and underwent an autopsy at the UFRA Pathology Laboratory. All procediments were consented by the animal owner.

In the necroscopic examination, the animal displayed a grade 3 body condition, pale ocular and buccal conjunctiva, congested lungs and liver with haemorrhagic areas,

splenomegaly with multiple whitish nodules on the surface, kidneys with dilation of the renal pelvis, and lymphadenopathy of the submandibular lymph nodes pre-scapular, axillary, inguinal and popliteal. In the large intestine, we observed bloody mucosa and the presence of numerous adult helminths in the colon, rectum, and anal mucosa. The lymph node and intestine samples were cleaved and processed for haematoxylin and eosin staining and histopathological diagnosis.

Our histopathological analysis unravelled alveolar oedema, dilated vessels in the liver, and the diagnosis of lymphoma in the spleen and lymph nodes. Histologically, these organs exhibited neoplastic lymphocyte cell proliferation with anisocytosis and an increase in lymphoblasts, distributed throughout the lymph node tissue, making cortico-medullary limit observation impossible with no perception of medullary cords and sinuses. Furthermore, we observed neoplastic lymphocyte infiltrations in the capsule and perilymph node fat as well as in the blood and lymphatic vessels.

In the mucosa of the large intestine, we detected helminthic forms in small cysts. The epithelial segments displayed no evidence of any inflammatory reaction. In a second section helminthic forms, including eggs, were immersed in the mucosa in the autolytic maceration area (Figures 1B, and C). Nevertheless, we detected capillaries distended by blood (congestion +) on the liver. The hepatocytes contained brown microgranular pigment (i.e., a lipofuscin characteristic), considering the age of the patient.

In total, we recovered 528 adult nematodes from the large intestine (Figure 1A). These nematodes exhibited an elongated and filiform anterior end with the presence of stichocytes in the oesophagus and a shorter and thickened posterior part (Figure 1D). Morphologically, the males displayed a folded or curved posterior with spikes in the proximal part and a smooth surface in the distal part, containing a spicule in the male specimens, while the posterior region of the females had a nearly straight end of the tail,

also containing a vulvar area (Figure 1E), uterus, and eggs in the internal region of the body (Figure 1F). Morphometrically, we analysed 20 eggs with an average length and width of 72.11 and 36.57 µm, respectively. The morphological and morphometric data were compatible with those of the species *T. vulpis*.

DISCUSSION

The most common parasitic helminth infections in dogs are those caused by *Toxocara canis* (Werner 1782), *Ancylostoma caninum* (Ercolani, 1859*)*, and *T. vulpis* resulting in haematological changes such as anaemia, eosinophilia, leucocytosis, and lymphocytosis (Dracz *et al*., 2014). The clinical signs and anatomopathological findings of this case demonstrate a multicentric lymphoma, weakening the immune system and worsening parasitic infectious conditions (Vail *et al.*, 2007; Ribeiro *et al*., 2017), resulting in a hyperinfection of adult nematodes in the large intestine. The morphological and morphometric characteristics of the nematodes and eggs were compatible with those of the species *T. vulpis* (Kagei *et al*., 1986). This infection in domestic dogs is common in peri-urban and rural areas, indicating a prevalence of over 40% and infections of up to 181 specimens per host in previous records (Ramos *et al*., 2015).

The patient, in this case, was presented with hypochromic anaemia and mild thrombocytopenia, which could be caused by tumour cell invasion into the bone marrow or immune-mediated platelet destruction, resulting from lymphoma. However, *T. vulpis* infection could have contributed to the blood loss (Ettinger *et al*., 2003; Bergman *et al*., 2007). Oedema in the limbs of the patient was a consequence of lymphadenopathy-related lymphatic flow obstruction (Cardoso *et al*., 2004). *T. vulpis* infection could have participated to the electrolyte and haematological imbalance (Venco *et al*., 2011), as adult *T. vulpis* nematodes secrete enzymes with cytolytic and haemolytic activities

(Kikuchi *et al*., 1964; Kirkova *et al*., 2005), beyond bacterial invasion through the epithelium of the large intestine (Schachter *et al*., 2020).

Haematological changes (e.g., eosinophilia and anaemia) and gastrointestinal pathologies (e.g., diarrhoea, abdominal pain, tenesmus, and rectal prolapse) could be observed in domestic dogs with trichurosis, mainly in rural areas with a tropical climate, where the lack of healthcare for small animals and the soil humidity and temperature conditions are optimal for the development of infective larval forms (Unterköfler *et al*., 2022; Borras *et al*., 2023). We highlight that the species *T. vulpis* and *Trichuris suis* Roederer, 1761 present cases of zoonosis (Areekul *et al*., 2010; Phosuk *et al*., 2018), causing mild diarrheal gastrointestinal conditions and even intestinal ulcers in human patients. Therefore, parasitic agent control and treatment in domestic animals that accompany humans is important (Dunn *et al*., 2002; Márquez-Navarro *et al*., 2012; Mohd-Shaharuddin *et al.,* 2019).

Although the type of antiparasitic medication applied ante mortem by the owner is unknown, this medication did not exert the expected effect on the patient, either because it was not specific to the parasitic agent or due to underdose. Helminth type-specific applications and doses are relevant in the case of serious infections. Moxidectin and fenbendazole treatment in severe *Strongyloides stercoralis* Bavay, 1876 infection cases in dogs did not result in the elimination of adult parasites and larvae, but the expected effect could be achieved when applying ivermectin (Unterköfler *et al*., 2022).

In this study, we reported a *T. vulpis* hyperinfection case, drawing attention by its unusual occurrence. Clinical severity must be considered in cases when the patient displays immunodeficiency, potentially associated with neoplastic disease, as in the case of lymphoma. A routine coproparasitological diagnosis and the periodic application of

specific antiparasitics for helminth and protozoan species would be considered important, especially in vulnerable patients such as older dogs.

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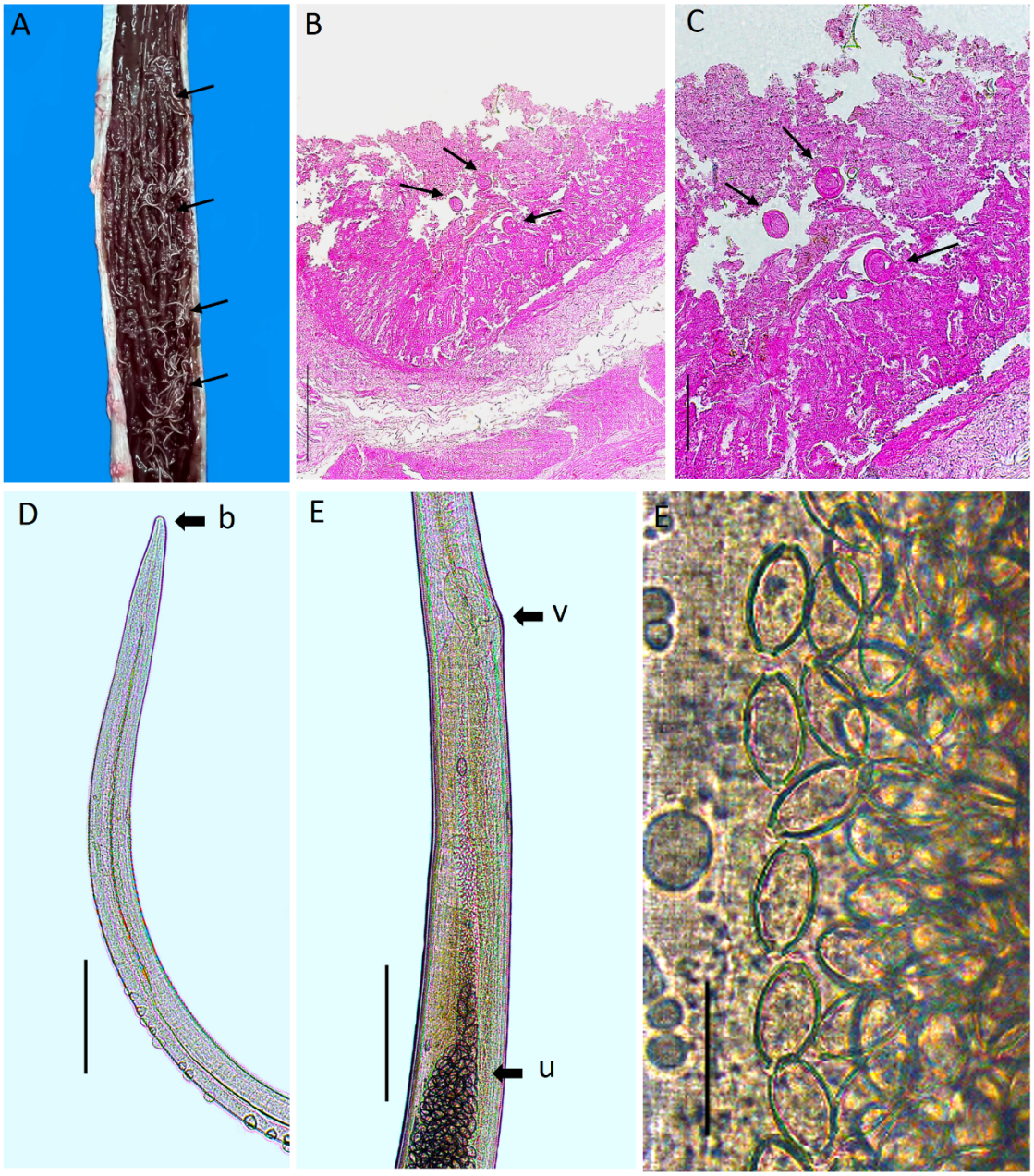

Figure 1. *Trichuris vulpis* hyperinfection: A) Numerous adult specimens in the bloody mucosa of the large intestine (arrows). B-C) Two adults segments in large intestine from histopathological section of the mucosa (arrows). D) anterior end of adult female showing cuticular processes (p), scale bar=500 µm. E) Vulvar opening in the middle region of the body (v) and uterus filled with eggs (u), scale bar=500 µm. F) detail of eggs inside the uterus, scale bar=100 µm.